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NOTE

Polymer-Protein Interactions: Changes with Tacticity

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Proteins interact with polymer surfaces to form apparent monolayer.[1-9] When protein-adsorption occurs on using a mixture of proteins (*i.e.*, plasma), the composition of the adsorbed layer appears to depend on the nature of the polymer surface in terms of a certain selectivity, rate, and concentration. These surface properties are often sensitive to processing and fabrication variables and usually become the main reason of their limited use in vascular repair and extracorporeal devices. Therefore, to understand how a particular polymer surface interacts in a particular environment, it is essential to understand the chemical nature of the surface with respect to its suitability inside the body in physiological environment by analyzing the interaction processes with blood proteins. In this note, we have attempted to investigate how the tacticity of polymers may effect the protein adsorption process and, thus its suitability as a blood compatible material.

EXPERIMENTAL

The block copolyether urethane ureas used in this study were synthesized[6] by using the solution polymerization method. These polymers were based on the poly(propylene glycol)s of the following molecular weights: 425, 710, 1010, 1025, 2025, ISO-1990,ISO-1580, ISO-1010, A-1920, A-1370, A-840, hard and soft (ISO-PPO) polymer (depending upon weight percentage of soft segment as listed in Table I), etc. ISO and A signify isotactic and atactic regularity respectively. Characterization of the surface of these polymer series and the difference between the air-glass side and air-air side of the polymers has already been done and discussed elsewhere[6,9] with other details including the film casting procedure, where a ten percent solution of polymer was prepared in *N,N*-dimethylformamide (DMF), (in DMSO for hard polymer). The size (3 cm × 2 cm) and surface area of the film was kept uniform ($\sim 12.0\,cm^2$).

Adsorption Work

A small glass bottle was weighed with and then without plasma (~ 20 mg). The plasma contained I^{125} radiolabeled desired proteins in calculated amounts. It was assumed that labeled protein and the proteins inside the plasma take part during the adsorption process exactly in the same way. Ten ml of the scintillation solution was added to the plasma weighed above, and the number of counts in 10 minutes was checked by a liquid scintillation counter. Average background counts (*i.e.*, number of counts for scintillation solution (10 ml) without plasma) were subtracted to get the counts due to plasma and thus the amount of plasma was calibrated in terms of number of counts. If the concentration of individual protein in plasma and the amount of radiolabeled protein added are known,

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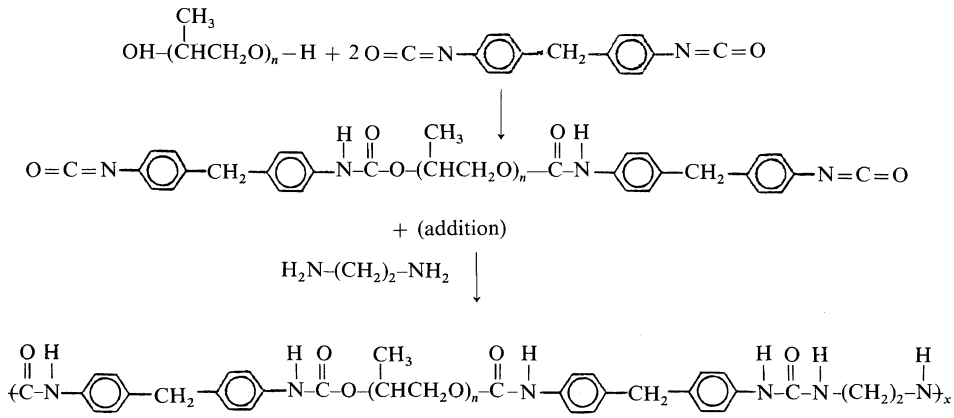

Table I. Adsorption of protein on static polymer surfaces

	wt% of soft segment	Protein	Amount of adsorbed protein/$\mu g\,cm^{-2}$				
			Adsorption time				
	%		10 min	30 min	60 min	120 min	180 min
Hard-polymer	11.9	Al.	0.00	—	0.02	0.00	0.10
KS-52		γ-G.	0.11	—	0.10	0.17	0.16
(air-glass)		F.		—		0.05	0.06
Com[a]-425-PU	51.3	Al.	0.00	—	0.06	0.08	0.23
KS-30		γ-G.	0.26	—	0.29	0.44	0.44
(air-glass)		F.	0.05		0.07	0.09	0.10
Com-710-PU	58.3	Al.	0.53	—	2.37	3.52	4.55
KS-27		γ-G.	0.31	—	0.62	1.13	1.20
(air-glass)		F.	0.33	—	0.85	1.25	1.56
Com-1025-PU	63.9	Al.	1.65	—	4.99	7.28	9.07
KS-20		γ-G.	0.36	—	1.08	1.52	2.10
(air-glass)		F.	0.64	—	1.71	2.23	2.96
Com-1010-PU	65.2	Al.	1.65	—	5.51	7.87	10.74
KS-31		γ-G.	0.43	—	1.46	1.44	1.97
(air-glass)		F.	0.85	—	2.12	2.71	3.97
Com-2025-PU	78.3	Al.	3.77	—	10.79	—	21.99
KS-29		γ-G.	1.00	—	2.48	4.94	6.22
(air-glass)		F.	1.43	—	3.65	—	7.51
Soft polymer	100	Al.	1.38	—	3.63	4.39	4.66
iso-ppo		γ-G.	0.28	—	0.28	0.43	0.39
(air-glass)		F.	0.06	—	0.05	0.07	0.05
Hard polymer	11.9	Al.	0.06	0.16	0.65	—	—
(air-glass)		γ-G.	0.28	—	0.25	0.33	—
		F.	0.00	—	0.03	0.06	0.07

Table I (continued).

	wt% of soft segment	Protein	Amount of adsorbed protein/$\mu g\,cm^{-2}$				
			Adsorption time				
	%		10 min	30 min	60 min	120 min	180 min
A-840-PU	60.0	Al.	0.90	1.52	3.39	—	—
(air-air)		γ-G.	0.47	0.60	0.62	0.84	—
		F.	0.11	—	0.38	0.67	—
A-1370-PU	71.0	Al.	5.78	7.49	7.76	—	—
(air-air)		γ-G.	0.63	—	1.14	1.35	—
		F.	0.26	—	0.87	1.33	—
A-1920-PU	77.4	Al.	improper films				
(air-air)		γ-G.	0.62	1.10	1.44	2.05	—
		F.	0.40	—	1.12	1.75	—
ISO-1010-PU	64.3	Al.	5.91	4.85	4.41	—	—
(air-air)		γ-G.	0.56	1.00	0.91	1.38	—
		F.	0.04	—	0.46	0.76	—
ISO-1580-PU	73.8	Al.	5.65	8.35	9.20	—	—
(air-air)		γ-G.	0.62	0.89	1.42	1.59	
		F.	0.39	—	0.91	1.57	
ISO-1990-PU	78.0	Al.	3.79	4.79	4.06	—	—
(air-air)		γ-G.	0.61	0.80	—	1.36	—
		F.	0.21	—	0.65	1.42	—
Soft polymer	100	Al.	1.00	3.65	5.89	—	—
iso-ppo		γ-G.	0.62	—	0.60	0.48	—
(air-air)		F.	0.01	—	0.03	0.03	0.04

[a] Com- based on commerical poly(propylene glycol)s (Union Carbide Corporation)[6].

the total protein can be evaluated from the number of counts.

Plasma was equally distributed in several beakers (depending upon the number of different polymer films) and several bottles with 10 ml of scintillation solution were prepared. First, the polymer film was placed in plasma for a definite period of time and then the film was taken out, rinsed with distilled water and put in the bottles with the scintillation solution. The number of counts was recorded by a liquid scintillation counter. The average background counts were subtracted and the particular amount of protein adsorbed was calculated in each cace.

Experiments were repeated for albumin, γ-globulin, and fibrinogen. Adsorption properties were evaluated in each case with polymers of various molecular weights and tacticity.

Experiments were also carried out with polymer films coating both sides of the glass plate, so that both air sides could come in contact with the proteins. The surface area in this case was $\sim 6.8\,cm^2$.

RESULTS AND DISCUSSION

Adsorption of three different proteins (albumin, γ-globulin, fibrinogen) on several polymer surfaces is given in Table I. The high energy sites of the polymer surfaces are assumed to be filled before other sites having higher entropies until saturation is reached. In other words, as soon as the polymer surface comes in contact with blood proteins, the polymer surface is modified due to protein adsorption which is dependent on the nature and distri-

bution of chemical groups on the polymer surface, since these chemical groups may have different affinities for interacting with different proteins depending upon their nature as polar or nonpolar. Therefore, protein binding may vary accordingly at a faster rate in the beginning until saturation is reached in the process of lowering the interfacial tension. The rate and adsorbed amount is dependent on both the protein and the polymer. Generally, on all surfaces, under experiment, albumin adsorbed faster than other proteins, however in some cases, such as hard polymer (KS-52) and Com-425-PU (KS-30), the surfaces adsorbed γ-globulin faster than albumin, and also in a few other cases, *e.g.*, Com-series the amount of adsorbed fibrinogen was larger than that of γ-globulin. These variations must be due to distribution of the polar–nonpolar groups on the surface. The exact mechanism for this is not yet known, but research is under way to clarify how preferential adsorption of one protein compared to the other occurs on various polymer surfaces.[6,9]

In our case, it seems that albumin, being a relatively smaller molecule (MW 69000) seems to be affected more in competition for adsorption on polymer-surfaces from plasma in our experiment, when all proteins are present. Further decreased adsorption of γ-globulin (MW 160,000 to 180,000) and fibrinogen in most cases, (MW 340,000) may possibly be due to greater bigger size or increased positive charge compared to that of albumin or both. On the basis of previous studies[10] it appears that the rapid adsorption of albumin may cause a reduction on the number of adhered platelets and probably be more thromboresistent. The experiments in this report provide an approximation of *in vivo* phenomenon and due to the complexity of the events at the interface they serve as an effective model only for understanding the process.

In general an increased amount of adsorption is observed as the weight percentage of soft segment in the polymer increases in the case of air-glass side samples. However, in the case of air-air side samples, similar behavior is observed for atactic polymers, while a decreasing amount of adsorption is observed in isotactic polymer, when the weight percentage of soft segment is above 74%. This indicates that the adsorption process is affected by the regularity of structure and molecular weight of the polymers. Therefore, one requires an indepth knowledge about any polymer intended for use in surgical implantation, since minor surface variation may cause significant differences towards blood compatible properties and this alone is the object of this note.

Although the experimental results presented here are not complete, we believe, however, that they are sufficient for demonstrating the relative changes discussed above.

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